NOVEL HAND-HELD DEVICE FOR EXHALED NITRIC OXIDE-ANALYSIS IN RESEARCH AND CLINICAL APPLICATIONS

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ABSTRACT. Changes in expired nitric oxide (NO) occur in airway inflammation and have proved to be important in the monitoring of inflammatory disease processes such as asthma. We set out to develop a novel hand-held NO-analyzer with a performance comparable to the present more costly and complex chemiluminescence instruments. The new device is based on a specially designed electrochemical sensor, where we have developed a novel sampling and analysis technology, compensating for the relatively slow response properties of the electrochemical sensor technique. A Lowest Detection Limit in NO-analysis from reference gas tests of less than 3 ppb and a response time of 15 seconds together with an average precision in human breath measurements of 1.4 ppb were obtained. We also show an agreement with the existing 'gold standard' FENO measurement technique, within 0.5 ppb in a group of 19 subjects together with a high linearity and accuracy compared to reference gases. The new analyzer enables affordable monitoring of inflammatory airway diseases in research and routine clinical practice.

KEY WORDS. chemiluminescence, electrochemical sensor, FENO, asthma monitoring.

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BACKGROUND

The chemically simple molecule NO, i.e. nitric oxide/nitric monoxide, has gained an increasing interest during the last two decades of medical research. It is involved in neurotransmission, blood pressure regulation, immune defence mechanisms and airway inflammation. The highly reactive property of nitric oxide in biological tissue makes a direct measurement of tissue NO difficult. Therefore, the measurement has often been made by indirect determination of L-citrulline, a metabolite produced when NO is formed from the amino acid L-arginine. Alternatively, it could be indirectly determined via the stable breakdown products nitrate NO_3^- or nitrite NO_2^-, i.e. oxidized metabolites of NO, when it is synthesized within cells via either of three NO synthase (NOS) enzymes from the corresponding encoding gene.

Since the finding in 1991 that nitric oxide is present in exhaled air of animals and humans (1), and the subsequent finding in 1993, that the level of exhaled nitric oxide is elevated among asthma patients (2), a major focus has been to further investigate and develop the clinical application of nitric oxide measurements in exhaled breath. Efforts have been focused on applying a non-invasive, simple and reproducible eNO (exhaled NO) measurement technique for use in diagnosis, compliance monitoring and efficacy control of anti-inflammatory treatment, and

potentially also dosage of anti-inflammatory drugs. Several modalities for measuring the concentration of eNO have been suggested. These modalities include tidal breath collection, multiple breath sampling and single breath tests with or without breath holding. The presently most widely accepted method is the Fractional Exhaled Nitric Oxide (FENO) method, which is defined in guidelines agreed by professional societies in Europe and the US (6, 7). FENO measurements in bronchial asthma have shown a strong correlation with inflammatory markers in bronchial biopsy, broncho-alveolar lavage and induced sputum investigations (3, 4, 5). For routine clinical handling of asthma patients, FENO measurement is the first objective way to monitor and diagnose airway inflammation.

RECOMMENDED METHOD FOR EXHALED NO MEASUREMENT

According to the guidelines for FENO measurement in adults (6, 7), a single breath sample is instantly analyzed as the subject performs a breathing manoeuvre, in which the subject makes an inhalation to total lung capacity (TLC) with scrubbed air, not to contaminate the sample with possibly high NO from the environmental air and then exhales for 10 seconds at a specified 5–20 cm H_2O counter pressure. This latter demand is to ensure soft palate closure, minimizing the risk of contamination of the exhaled gas from nasal NO originating from the paranasal sinuses, where NO concentration levels are 10-fold to 1000-fold higher than in the lower airways (8). In children the exhalation time can be shorter. The guidelines also recommend an exhaled flow of 50 ml/s (FENO$_{0.05}$), based on the hypothetical assumption that the region of interest for the NO excretion is within the lower noncartilaginous, but not the most peripheral, parts of the airways, i.e. excluding the sections close to and including the alveolar space. This relates to the reasoning that the airways are considered similar to a basic tubing system through which the expired air is led. If there is no NO depletion within the airway walls during the air passage, a steady state condition and thereby a stable exhaled concentration level (plateau) is reached, corresponding to the chosen exhalation flow rate. The exhalation flow rate has great methodological influence on the exhaled concentration level, with low flows resulting in higher levels and vice versa (8).

A normal NO concentration in humans (FENO$_{0.05}$) not suffering from airway inflammation is in the range 10–20 ppb in adults, somewhat lower (5–25 ppb) in children (9). In asthma patients, who are not accurately treated with anti-inflammatory medication, the exhaled NO values can reach concentrations well exceeding 100 ppb.

AVAILABLE TECHNOLOGIES

NO-measurements are common in atmospheric and environmental research. Apart from mass spectrometry, sometimes in combination with gas chromatography, the generally accepted 'gold standard' gas phase NO analysis method since decades, has been the chemiluminescence method. The obvious choice for measurements of eNO in the early 90's was therefore to apply the chemiluminescence technique also here (1, 2).

With the chemiluminescence technique, NO molecules are detected on the basis of radiation created from a chemical reaction. Ozone (O_3) is generated in the instrument and combined with the NO molecules in an air-stream sample led into an evacuated reaction chamber, so that excited nitrogen dioxide (NO_2^*) molecules are formed in the chamber. The subsequent shift to a lower energy state of the NO_2^* molecule emits electromagnetic radiation (photons), which can be detected as light by a photomultiplier tube. The resulting output signal is determined and corresponds linearly to the NO concentration in the sample, as long as O_3 is present in excess.

Generally, the mass of chemiluminescence analyzers range 25–45 kg and instrument prices range 20 000–45 000 USD. Performance characteristics for a representative chemiluminescence analyzer model in the market are listed in Table 1.

To give reliable measurement results compensating for the inherent drift, chemiluminescence gas analyzers have to be calibrated regularly, i.e. once daily or at least every fortnight according to the instrument manufacturer's recommendations. As the chemiluminescence instruments are considered highly linear in their optical bench behaviour, the recommended NO calibration gas is either chosen as a high concentration NO gas (e.g. around 50 ppm) or a concentration within the normal operation range of the analyzer, e.g. a low gas concentration of 200 ppb. There are several gas manufacturing companies for NO calibration gas on the market, but unfortunately few of them have shown a reliable and stable gas product according to their specifications over the whole expected shelf-life period, i.e. normally 6–12 months from manufacturing. The need for frequent calibrations with a gas with limited shelf life adds significantly to the total running cost of the chemiluminescence instruments.

Typically, the calibration procedure takes a few minutes to perform and the result is a manual or automatic baseline adjustment to the optical bench of the instrument. Certain limitations for accepted adjustment are normally applied in case of an automatic adjustment feature, not to loose track of the initial setting and adapt to the natural drift of the optical bench components and electronics.

Table 1. Key requirements on instrument parameters for chemiluminescence, electrochemical sensor NO gas analysis of existing and novel type, for use in exhaled breath measurement in clinical settings

Instrument parameter	Chemiluminescence (Examples from NIOX®)	Electrochemical (previous state-of-the-art)	Aims for the present development
Measurement range (ppb)	0–25 000 ppb	50/100–100 000 ppb	5–300 ppb
Sensitivity/Lowest Detection Level (ppb)	1.5 ppb	50–100 ppb	5 ppb
Resolution (ppb)	±0.1 ppb	±50–500 ppb	±1 ppb
Response time (10–90% response at 0–200 ppb NO gas range)	<0.7 seconds (NO analyzer 0.2 seconds)	30–120 seconds	<15 seconds
Linearity (0–200 ppb NO gas)	<2.5 ppb	3–5% (without re-calibration)	<1% (without re-calibration)
Agreement (with standard NO gas, 0–200 ppb range)	<±2.5 ppb	n/a	<±3 ppb (at 30 ppb or less) or <± 10% (above 30 ppb) (0–300 ppb range)
Repeatability/precision (with standard NO gas, 0–200 ppb range)	<2.5 ppb (expressed as SD for concentrations below 50 ppb or as coefficient of correlation (CV) for measured values above 50 ppb	n/a	<3 ppb (at 30 ppb or less) or <10% (above 30 ppb) (0–300 ppb range)
Drift	<±3 ppb / 14 days (0–200 ppb range)	n/a	<10% / 12 months (0–300 ppb range)
Specificity/Interference	<10% of measurement value at 5% volume CO_2 and 5% volume water	n/a	<3 ppb from O_2, CO_2, ethanol, NO_2, NH_3, etc.
Portability	On a cart	n/a	Hand-held
Operation temperature	+15 to +30°C	n/a	+16 to +30°C
Operation humidity	30 to 75% RH (non-condensing)	n/a	30 to 60% RH (non-condensing)
Operation pressure	860 to 1060 hPa	n/a	700 to 1060 hPa
Dimensions (H × W × D)	500 × 300 × 400 mm	n/a	250 × 150 × 100 mm
Weight	40 kg (including display monitor, excluding calibration gas cylinder)	n/a	<1 kg
Calibration demand	Recommended once every 14 days (or at drift beyond specification)	n/a	No re-calibration required during sensor life time
Calibration gas	200 ppb ± 10% 3 bar NO in N_2 having a 6–12 months life time	n/a	Not required
Measurement time (from breathing manoeuvre to presentation of FENO result	10 seconds (at default settings)	n/a	<2 minutes
Price level	40 000 USD	n/a	<4 000 USD

Regular technical service and adjustment is also needed according to each manufacturer's service interval recommendations. This type of service is normally not offered as a field service procedure by the suppliers and the instrument then has to be shipped to a dealer or support centre, where appropriate measurement instruments, gas regulators, etc. are available. This also increases the running cost as well as decreases the analyzer up time.

In summary, therefore the chemiluminescence analyzers in the market today show several drawbacks concerning investment cost level, user friendliness, transportability and requirements for frequent calibration and technical maintenance. Clearly, these obstacles have to be greatly reduced in order to achieve full acceptance of exhaled NO measurement methods in general routine healthcare, especially when moving into home monitoring.

AIMS OF THE PRESENT DEVELOPMENT

The purpose of the present work was to develop and test a simplified instrument for FENO monitoring, primarily for asthma patients in point-of-care locations. In order to provide an instrument for use outside the specified laboratory environment, the aim was an instrument, with a mass reduction at a factor of 40 and price by at least a factor of 10, compared to existing devices. More specifically, the aims are listed in Table 1, together with corresponding data of present devices and including existing versions of one of the candidate new sensor technologies.

POTENTIAL NEW SOLUTIONS

The potentially applicable sensor technologies were scanned and after an initial pre-study, the electrochemical sensor technology was chosen for further adaptation into a final instrument concept. To be able to use a new analysis technology for clinical FENO testing, the discussed basic performance parameters need to be fulfilled, including high precision and agreement down to an NO concentration of only a few parts per billion (ppbv) (Table 1).

Until now, the well-known electrochemical sensor technology has been used for e.g. measurements of environmental gases including NO at industrial applications and in environmental gas measurement, although only with less demanding requirements for measurements in concentration ranges from a few hundred ppb to ppm levels.

FEATURES OF A NEW NO ANALYZER

The device includes a sampling and gas conditioning system, an NO sensor and a man-machine interface (MMI)
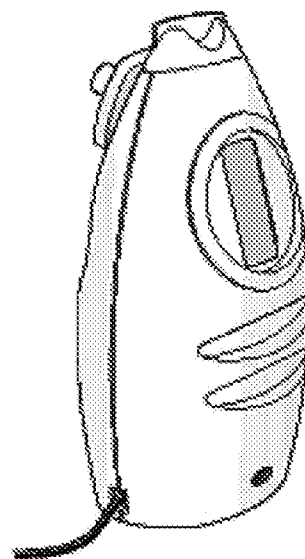

Fig. 1. The new hand-held NO analyzer. Dimensions are 240 × 130 × 100 mm.

in a compact housing (Figure 1). The user is guided on the built-in touch-screen display through the breathing manoeuvre by use of the interactive MMI. The valves and pumps of the instrument are automatically controlled to handle the inhaled sample appropriately via the instrument electronics and software program. The analyzed gas humidity exposed to the sensor is equilibrated to environmental humidity by having it passing a custom-designed drier assembly. NO levels in ambient air may sometimes be high and have been shown to influence the measurement value in single breath measurements (10), why the use of NO-free air filtering to an NO concentration of below 5 ppb is applied as recommended (7). Another important measurement control parameter is the exhalation flow during the exhalation procedure making it equal and standardized for all users. To give accurate and repetitive FENO measurements, a dynamic flow control unit for the recommended 50 ml/s BTPS (Body Temperature and Pressure Saturated, i.e. +37 °C, ambient pressure and 100% relative humidity) exhalation flow is used (7).

In order to achieve comparable measurement results, as when using the established standard chemiluminescence real time technique, there is a need to compensate for the slower dynamic response of the electrochemical sensor. This is obtained in the study instrument by the development of a buffering unit, allowing storage of the last portion of the exhalation sample. The sequentially stored sample is then transferred to the sensor via a pump and valve arrangement for analysis.

Sensor design and function

Any gas which can be electrochemically oxidized or reduced, can also be detected by means of an electrochemical sensor. The measurement principle, adapted in the electrochemical sensor for NO analysis of this novel instrument, is based on the well-known amperometric technique (11). Within a limited total pressure range, the output signal from the sensor is directly proportional to the partial pressure of NO in the sample, and thereby also to the concentration of NO in the sample.

An important characteristic of the newly developed sensor is its high sensitivity down to the level of a few ppb. The composition of the catalyst material, the electrolyte composition, a sophisticated arrangement of different diffusion-barrier forming membranes, as well as a specific chemical filter are all designed to maximize the NO selectivity and sensitivity from the complexly composed exhaled breath sample.

Apart from the visual display and interaction features, the instrument is also equipped with audible and alternative visual feedback to handling actions expressed during the breathing phase.

METHODS

Studies to evaluate technical sensor performance

In order to make performance comparisons of the novel instrument with the current 'gold standard' for exhaled NO measurements, we performed comprehensive tests on both a chemiluminescence based NIOX® Exhaled NO monitoring system (Aerocrine AB, Solna Sweden) and the new hand-held prototype instrument by studies on the agreement and precision, both with calibration gas and during FENO testing.

Comparison with reference gases

For reference gas measurements with the hand-held NO analyzer, the FENO procedure was partly simulated, so that the instrument was triggered by an external signal and the reference gas mixture was supplied during 10 seconds through the patient inlet filter and at the appropriate pressure. Agreement, precision and linearity determinations were made separately and each included 10 consecutive measurements at six different NO gas concentrations up to 218/219 ppb nominal.

The lowest detection limit was assessed in two representative instruments with 10 repetitions at the lowest available reference gas concentration. For all gas measurements on the new instrument and sensor, NO was diluted in nitrogen using a gas mixer (Environics model 400, Tolland, Connecticut, U.S.).

Interference with other compounds

As human exhaled air could consist of at least 200 different gaseous compounds interference from these compounds would be a serious measurement limitation. Investigations have shown large inter-individual concentration differences in humans in the multitude of different substances exhaled (12, 13). The potential performance-degrading interference from other confounding compounds with the sensor in the study instrument was investigated with measurements on all proposed relevant compounds, far above the expected maximum concentration in exhaled and environmental air, including the more abundant oxygen, carbon dioxide, nitrogen dioxide, isoprene, hydrogen peroxide, ethanol and ammonia.

Comparison with 'gold standard' instrument

Agreement study

Nineteen healthy volunteers performed FENO measurements with one hand-held instrument and one NIOX instrument. At each occasion subjects performed the FENO procedure once with each of the instruments during a total of 251 sessions.

Precision study

Nine healthy subjects performed triplicate FENO measurements with both instrument types. Subjects came at three occasions on consecutive days. For the hand-held instrument such triplicate tests were repeated with six different sensor units, in order to define performance variability.

Statistical analysis

The statistical analyses of the sensor and instrument at gas and FENO measurements including comparisons between the two NO-analyzers, were performed as mean calculations for agreement and as standard deviation (SD) calculations for precision investigations.

Ethics

The present studies were performed after application to the Reference Ethical Committee at Karolinska Sjukhuset

(KI forskningsetikkommitté Nord), Stockholm, Sweden, but were considered as part of the quality assurance procedures in product development. They were therefore deemed by the committee, not to require ethical committee approval and thereby excluded from the activities of the ethical committee. The Swedish Medical Products Agency (Läkemedelsverket) Uppsala, Sweden made the same conclusion. Informed consent was received from the participating subjects, who were all adults.

RESULTS

Mass

Apart from measurement and specification goals for the new instrument, a general weight reduction approach involving all parts of the instrument, resulted in a weight relation to the NIOX NO-analyzer of around 1:50, to approximately 0.8 kg.

Comparison with reference gases

Agreement and precision

Table 2 shows the observed gas concentration data from a representative sensor installed in the hand-held instrument.

Hence, the absolute mean error, i.e. difference of all measured concentrations presented above (from gas mixtures 5 ppb to the nominal gas concentration 218 ppb) was from 0.8 to 13.6 ppb, with an SD of 0.5 to 2.0 ppb.

Table 2. Repetitive reference gas measurements in the hand-held instrument with a representative sensor for NO concentrations 5, 10, 20, 50, 100 and 218 ppb, respectively

NO Concentration (ppb)	5	10	20	50	100	218
Measurement #						
1	1.3	0.5	0.9	0.0	−4.2	−16.8
2	2.0	1.7	0.7	−2.2	−3.5	−15.7
3	2.2	2.1	1.5	−2.9	−4.3	−15.2
4	1.6	2.3	1.2	−1.2	−3.8	−14.5
5	2.6	2.1	0.7	−1.0	−3.0	−13.7
6	2.7	3.4	0.0	−0.8	−3.6	−13.8
7	1.3	2.9	0.2	−0.1	−3.1	−12.6
8	1.9	2.9	1.1	−0.7	−3.3	−11.9
9	0.8	2.9	0.7	−0.5	−3.3	−11.9
10	0.7	2.7	1.1	−0.5	−3.0	−10.1
Mean difference (ppb)	1.7	2.4	0.8	−1.0	−3.5	−13.6
SD (ppb)	0.7	0.8	0.5	0.9	0.5	2.0

Data are differences (measured − nominal) for 10 consecutive measurements at each concentration.

Linearity

Linearity testing as investigated for a representative sensor from 10 repetitive measurements presented in Figure 2, reveal a satisfactory linear behaviour at the nominal NO gas mixture concentrations 5, 10, 20, 50, 100 and 219 ppb in N_2, with a mid-range deviation of 0.6%.

Lowest detection limit

LDL was tested by 10 consecutive measurements in two representative sensors and instruments, with the lowest available NO gas concentration of 2.5 ppb, showing a mean concentration of 2.4 ppb and a SD of 0.5 ppb.
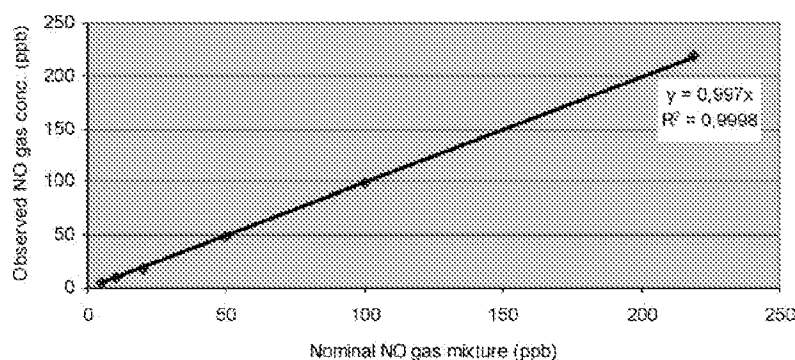

Fig. 2. Result from linearity test on a representative sensor and instrument with calibration NO gas mixtures (mean out of 10 consecutive measurements at each concentration).

Interference with other compounds

Data were obtained with representative sensors for a number of potential endogenous and environmental interference compounds. The acceptance criteria for the tests was an interference NO-equivalent of less than 3 ppb over the operating range, corresponding to 1% of the maximum measurement value, based on comparable recommendations from the American Thoracic Society (7). As the individual sensitivity to NO and interference vary between sensors, a multi-fold safety margin was added to the worst-case calculations. Using the above criteria, there was no significant interference.

FENO measurement comparison with 'gold standard' instrument

Agreement study

Table 3 shows the instrument agreement for 19 participating subjects. A total of 251 measurement sessions were conducted in this study.
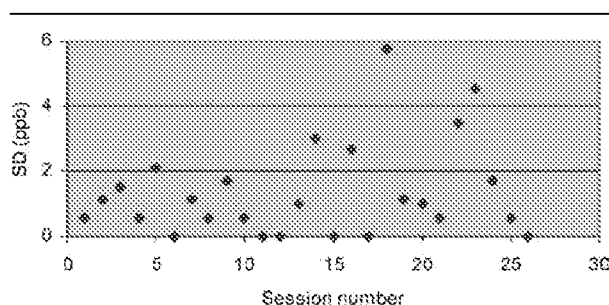

Fig. 3. *Precision data presented as the within-triplicate value SD from a representative sensor, where 9 subjects performed triplicate measurements in the hand-held NO-analyzer, repeated during three consecutive days (10-second exhalation).*

The data from the new NO-analyzer versus NIOX investigation presented above show an average disagreement of 0.5 ppb with a mean SD of 3.8 ppb, from totally 251 measurement sessions in the 19 subjects participating. Subjects who performed only one or two measurement sessions were excluded from the SD calculations.

Table 3. *Agreement between FENO tests with NIOX and the new hand-held NO-analyzer*

Subject #	Number of measurements performed	NIOX mean (ppb)	Hand-held NO-analyzer mean (ppb)	Hand-held NO-analyzer–NIOX mean difference (ppb)
1	12	12.1	11.8	−0.3
2	1	16.4	17.0	0.6
3	16	29.8	29.6	−0.2
4	5	15.2	12.6	−2.6
5	43	19.5	18.6	−0.9
6	1	3.2	5.0	1.8
7	3	10.4	10.3	−0.1
8	19	18.4	18.3	−0.1
9	8	7.4	6.5	−0.9
10	29	34.7	32.6	−2.1
11	31	26.3	26.1	−0.2
12	8	17.4	17.8	0.4
13	1	14.2	18.0	3.8
14	7	15.8	15.7	−0.1
15	12	13.6	13.2	−0.4
16	2	36.7	34.0	−2.7
17	40	15.2	15.1	−0.1
18	11	40.6	38.5	−2.1
19	2	10.8	7.5	−3.3
N	251			
Mean		18.8	18.3	−0.5

Values are from between 1 and 43 paired tests with the two devices performed by 19 subjects (10-second exhalation).
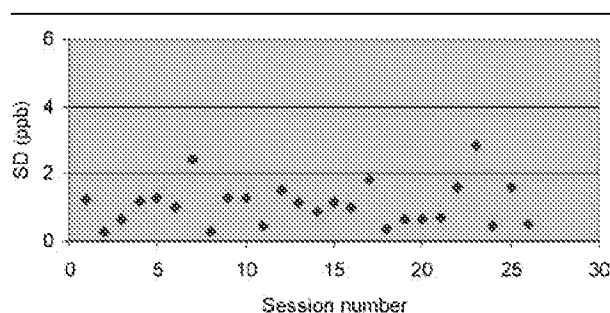
Fig. 4. Precision data presented as the within-triplicate value SD as obtained together with the data shown in Figure 3, when the subjects performed triplicate measurements in NIOX, repeated during three consecutive days (10-second exhalation).

Precision study

The precision study was performed to compare the precision in three consecutive measurements with the two hand-held instruments in nine subjects (10-second exhalation). Figure 3 shows data from a representative sensor.

The same sensor showed a precision expressed as average SD of 1.4 ppb. Data from 462 single measurements in the nine subjects and with six different sensors were identical.

Figure 4 shows the corresponding results with NIOX from the same subjects.

The precision expressed as average SD for the NIOX data of Figure 4 was 1.1 ppb. The corresponding SD value obtained in all sessions and subjects was 1.4 ppb.

CONCLUSIONS

The present tests of a novel device for exhaled NO measurements have shown that the key features of agreement, precision and linearity are comparable to the currently accepted chemiluminescence technique, thus fulfilling requirements for the intended clinical use. There is a few ppb disagreements from the corresponding 'gold standard' technique, but it should be recognized that also the chemiluminescence analyzers are afflicted with a certain measurement error in FENO samples, which hence is included in the performance data in these studies. Making the same type of gas measurements on NIOX and the new hand-held NO-analyzer, we reported the same precision level in repeated testing.

The functional comparison between the new hand-held device and the chemiluminescence instrument should also involve the marked reduction in mass and expected cost level of the new technique. In our view, the novel device is a major improvement with a large potential in clinical routine management of inflammatory airway diseases.

CONFLICT OF INTEREST STATEMENT

Tryggve Hemmingsson is an employee of Aerocrine AB Solna, Sweden and Rudolf Gambert is an executive of International Technologies Dr. Gambert GmbH, Wismar, Germany. Dag Linnarsson has no financial relationship with a commercial entity that has interest in the subject of this paper.

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